SYNOPSIS

Rotaviruses are the leading cause of acute diarrhoea in infants and young children worldwide causing over a million deaths annually in developing countries Rotavirus, whose genome consists of 11 double-stranded RNA segments, belongs to the family Reoviridae Rotaviruses have been classified into seven groups, A to G, based on group-specific antigens detected primarily on the intermediate capsid protein, VP6 Group A rotaviruses exhibit two commonly studied serologic specificities i e , subgroup and serotype and are subject to extensive studies as they constitute the majority of rotaviruses in man VP6, the intermediate capsid protein encoded by the 6th segment of viral RNA, is the subgroup-specific antigen At least four distinct subgroup (SG) specificities, i e , SGI, SGII, SGI/II and SG non (I/II), have been identified among group A rotaviruses The outer capsid proteins VP4 and VP7 specify two independent serotype specificities, i e , the P type (for protease-sensitivity of VP4) and G serotype (for glycoprotein nature of VP7), respectively In contrast to P serotype characterization, rotaviruses from both humans and animals have been well classified into at least 14 G serotypes

Also, rotaviruses in the clinical samples are identified and characterized extensively by comparison of the migration patterns of viral genomic RNAs by polyacrylamide gel electrophoresis All Group A rotaviruses can be classified into two distinct electropherotypes exhibiting either 'long' or 'short' RNA pattern in which the migration of segment eleven is fast or slow, respectively Moreover, human rotaviral strains with serotype 2 or 8 specificity exhibit 'short' RNA electropherotypes, while serotype 1,3,4 and 9 strains have 'long' RNA electropherotypes It has also been observed that the vast majority of human rotaviruses with SGI specificity have a 'short' RNA pattern where as those with SGII specificity have 'long' RNA pattern On the other hand, the great majority of animal strains appear to have a 'long' RNA pattern but SGI specificity Therefore, the occurrence of rotaviruses with 'long' RNA pattern and SGI specificity in humans is an indication of the likelihood of their being derived from animals In recent years several strains of human rotavirus have been isolated that exhibit

SGI specificity but 'long' RNA pattern These unusual strains exhibit either a known or a new serotype specificity and cause diarrhoea in children Some of these strains appear to be genetically related to rotavirus strains predominantly found in cats and dogs

While rotavirus infection in young children below 5 years of age often causes severe diarrhoea, infection in newborns is often asymptomatic Earlier studies have indicated that neonatal human rotavirus infections may confer protection against subsequent development of diarrhoea caused by symptomatic viruses Based on the hypothesis that neonatal strains represent naturally attenuated viruses, several investigators have proposed the neonatal strains as vaccine candidates The extensive morbidity and mortality associated with rotavirus diarrhoea worldwide, particularly in developing countries, has made the development of a rotavirus vaccine a high priority programme

The major objectives of this study were

i To study the epidemiology of asymptomatic and symptomatic infections in Bangalore and Mysore during a seven-year period from 1988 to 1994 to understand the antigenic nature (serotype) of Indian rotaviruses,

ii To characterize the gene encoding the SG-specific antigen VP6 of the IS2 (symptomatic) strain and I321 (asymptomatic) strain of rotavirus to serve as nucleic acid probes for detection of rotaviruses as well as to determine genetic variation in VP6 from Indian strains,

iii To express VP6 from the symptomatic strain IS2 in *E coli* and production of antiserum against the expressed VP6 towards the goal of development of reagents for a recombinant vaccine in conjuction with VP4 and VP7 as well as to study the structure of VP6

The results and conclusions are briefly summarized below

1) ***Epidemiology of asymptomatic infections***

A large number of samples were collected from healthy newborn infants (2

to 30 days old) from six general hospitals in Bangalore and one general hospital in Mysore All the samples were analysed for their RNA migration pattern (electropherotype), SG and serotype All the isolates exhibited a 'long' electropherotype and a SGI specificity Strains collected from hospitals separated by 5 to 10 km showed similar characteristics These strains exhibited serotype 10 specificity Rotaviruses belonging to serotype 10 are predominantly found only in cattle and have never been previously identified in healthy neonates Samples from Bangalore and Mysore showed similar characteristics As they efficiently infect and replicate in humans, grow well in cell culture and are naturally attenuated, I321-like asymptomatic strains represent the best candidates available so far for a 'Jennerian' type live rotavirus vaccine either on their own or as reassortants

2) *Epidemiology of symptomatic infections*

Stool samples were collected from young children between 3 months to 5 years of age from 1988 to 1994 in Bangalore and 1993 to 1994 in Mysore These samples showed either a 'long' or a 'short' pattern of migration A significant observation was that the incidence of rotavirus infection in Bangalore was found to decrease from 45 3% in 1988 to 1 8% in 1994 This is in contrast to asymptomatic infections which showed a frequency of about 34% throughout the study period This drastic reduction in rotavirus infection over a period of time has not been reported anywhere The reason for this reduction is not clear It is likely that this reduction in the number of gastroenteritis cases in Bangalore is due to cross-protection effected by asymptomatic infections of neonates by I321-like strains While strains belonging to SGII were found to be more predominant than those having SGI specificity, seven strains with dual SG specificity were also observed In Bangalore serotypes G2 and G3 were found to be predominant during 1988 to 1990 Serotypes G1 and G3 were more frequently observed during 1991 and 1992 while serotype G2 and G4 were less frequent or absent During the two-year study in Mysore, serotypes G1 and G3 were predominant while serotype G2 and G4 were completely absent

Viruses with serotype 10 specificity were not detected in symptomatic infections indicating that the I321-like asymptomatic strains are not involved in symptomatic infections Several strains with SGI specificity and a 'short' pattern which did not show serotype 2 specificity were identified These strains either showed high reactivities with all the serotype-specific monoclonal antibodies or showed very low reactivities About 36% of the samples from Bangalore and 30% samples from Mysore could not be assigned any serotype Some of these strains may belong to uncommon serotypes like G8 and G9 or represent strains of unknown serotype specificity

The results from these epidemiological studies indicate that antigenically distinct rotaviruses are circulating in India and this fact should therefore be taken into account while designing vaccine strategies under the Indian context

3 ***Cloning and sequence analysis of gene 6 from symptomatic strain IS2 and asymptomatic strain I321***

Gene 6 encoding VP6 is the most conserved gene among group A rotaviruses from humans as well as animals and is therefore a potential candidate for use as a DNA probe for virus detection Also, because VP6 is the most predominant protein (>50%) of the virion, it is the antigen of choice for detection of rotaviruses by ELISA To date, there is no information on the extent of genetic and/or antigenic variation in gene 6 from Indian rotaviruses In this study, gene 6 from the prototype symptomatic strain IS2 and asymptomatic strain I321 were cloned and sequenced Both the genes were found to be 1356 bp in length and showed higher homology to bovine rotaviruses rather than human rotaviruses These studies confirmed that gene 6 of I321 is also derived from the bovine virus Interestingly, the symptomatic IS2 strain belonging to serotype 2 also contained gene 6 that is more closely related to bovine rotavirus indicating that human serotype 2 strains might have arisen due to reassortment between a bovine and an yet unknown progenitor rotavirus

VP6 from IS2 strain showed substitution by lysine at two amino acid positions 97 and 134 Amino acids at these two positions are highly conserved in VP6 from all other group A rotaviruses VP6 from IS2 strain is consequently more basic than that from other group A rotavirus strains Since VP4 from IS2 strain is more acidic, due to acidic amino acid substitutions, than the VP4 alleles seen in other serotype 2 viruses, these complementary changes might be essential for stabilizing the interactions between these outer and intermediate shell proteins

4 ***Expression of VP6 from IS2 strain of rotavirus***

VP6 from the IS2 strain was expressed in *E coli* using the pET20b(+) expression vector The recombinant VP6 expressed from this vector migrated as two distinct bands of 50 4 kDa and 48 0 kDa due to inefficient cleavage of the pelB leader sequence Deletion of this leader sequence resulted in the expression of a protein of molecular weight 46 4 kDa Smaller protein products of 34 kDa, 24 kDa and 23 kDa were also detected which might be cleavage products or may have arisen due to internal initiation of translation within the gene 6 sequence

The recombinant VP6 was purified by single-step affinity chromatography and polyclonal antiserum was raised against it in rabbit Recombinant VP6 was recognized by polyclonal antiserum as well as SGI-specific monoclonal antibody that recognizes conformational epitopes indicating that VP6 expressed in *E coli* has native conformation The production of recombinant VP6 constitutes a part of the efforts directed towards understanding the structure of rotavirus encoded proteins as well as development of reagents for a comprehensive vaccine against rotavirus gastroenteritis under the Indian context